Analysis of mutations of *rpoB* gene in *Deinococcus radiodurans* R1 induced by simulated space conditions

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To investigate the microbial viability and their DNA damage, the radioresistant bacteria *Deinococcus* spp. have been exposed at Exposure Facility of International Space Station (ISS) in Tanpopo mission since May 2015 [1]. The Exposure Panels (EPs) harboring dried-deinococcal cells will return to the ground after about one-, two- and three-year exposure. We are going to analyze the survival rate and DNA damage of dried deinococcal cells using pulsed-field gel electrophoresis, quantitative-PCR and mutation assay. The antibiotics rifampicin binds the RNA polymerase β-subunit, which is encoded by *rpoB* gene, and inhibits the initial step of the transcription activity. Certain mutations in the *rpoB* gene confer rifampicin resistance [2]. Based on the above understanding, we will determine mutant frequency and the mutation spectrum for the *D. radiodurans rpoB* gene. From these mutation data, we will estimate major DNA damage induced by space environment. For this purpose, the mutagenic specificity of the *D. radiodurans rpoB* gene in simulated space conditions was investigated in this study.

The *D. radiodurans* R1 cell-suspension was dropped in the wells of aluminum plates (φ20 mm) and was dried under vacuum (vacuum-dried). The dried cells were exposed to vacuum (< 10\(^{-5}\) torr) or UVC\(_{254nm}\) under the vacuum conditions. As a control, we analyzed the vacuum-dried cells without additional vacuum incubation. After exposure experiment, the cells were recovered from each well, inoculated into 10 ml of mTGE medium and cultured to show the OD\(_{590}\) nm to be about 4. The cell suspension was plated on mTGE agar containing 50μg/ml of rifampicin to determine the number of rifampicin resistant colonies (Rif\(^R\)), and on mTGE agar without rifampicin to determine the total number of viable colonies.

The rifampicin-resistant mutant frequency of vacuum-dried cells was 1.3 (±0.5) x 10\(^{-8}\). The rifampicin-resistant mutant frequency of the *D. radiodurans* R1 wet cells has been shown to be about 1.5 x 10\(^{-8}\) [3]. The result suggests that the rifampicin-resistant mutant frequencies of vacuum-dried cells and wet cells are comparable for *D. radiodurans* R1. Further, we will report and discuss the rifampicin-resistant mutant frequency and mutation spectra in the *rpoB* gene of rifampicin-resistant cells following exposure to UVC\(_{254nm}\) and vacuum (< 10\(^{-5}\) torr).


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